

Claims

1. An immunoglobulin produced by recombinant host cells.
- 5 2. An immunoglobulin substantially free of other proteins with which it is normally associated in vertebrate cells.
- 10 3. The immunoglobulin of Claim 1 or 2 which is a mammalian antibody, in that the amino acid sequences of all four chains are homologous to the sequences in the corresponding chains in an antibody derived from a mammalian species.
- 15 4. The antibody of Claim 3 which is directed against CEA.
6. The antibody of Claims 3 wherein the light chain is of the kappa family.
- 20 7. The immunoglobulin of Claim 1 or 2 which is a hybrid antibody.
8. The immunoglobulin of Claim 1 or 2 which is a composite non-specific immunoglobulin.
- 25 9. The immunoglobulin of Claim 1 or 2 which is a chimeric antibody.
10. The antibody of Claim 9 wherein the constant regions of all four chains are homologous to the corresponding constant regions of an antibody of a first mammalian species, and the amino acid sequence of the variable regions of all four chains are homologous to the variable regions in an antibody derived from a second, different, mammalian species.

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11. The immunoglobulin of Claim 1 or 2 which is an altered antibody.
12. A composition of matter consisting essentially of a univalent antibody.
- 5 13. The composition of matter of Claim 12 which is produced by recombinant host cells.
- 10 14. A composition of matter consisting essentially of Fab protein.
- 15 15. A composition of matter of Claim 14 which is produced by recombinant host cells.
16. The composition of matter of Claim 13 or 15 which is mammalian.
- 15 17. The composition of matter of claim 13 or 15 which is immunoreactive against CEA.
18. A sequence of amino acids produced by recombinant host cells corresponding to immunoglobulin heavy chain.
- 20 19. The sequence of Claim 18 which is a mammalian heavy chain.
- 25 20. The sequence of Claim 19 which is anti-CEA heavy chain.
21. The sequence of Claim 19 which is a chimeric heavy chain.
22. The sequence of Claim 21 wherein that portion of the sequence which corresponds to the constant region is homologous to corresponding sequence of an antibody derived from humans, and the amino acid sequences of the variable region is homologous to the corresponding amino acid sequence of an antibody derived non-human mammalian species.

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23. A sequence of amino acids produced by recombinant host cells corresponding to immunoglobulin light chain.
24. The sequence of Claim 23 which is a mammalian light chain.
- 5 25. The sequence of Claim 23 which is anti-CEA light chain.
26. The sequence of Claim 23 which is a chimeric light chain.
- 10 27. The sequence of Claim 26 wherein that portion of the sequence which corresponds to the constant region is homologous to corresponding sequence of an antibody derived from human, and the amino acid sequence of the variable region is homologous to the corresponding amino acid sequence of an antibody derived from non-human mammalian species.
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28. A DNA sequence which encodes for the immunoglobulin of Claim 1 or 2.
- 20 29. A replicable expression vector capable of expressing in a suitable host cell the DNA sequence of Claim 28.
30. An expression plasmid which comprises the DNA sequence of Claim 28 operably linked to a promoter compatible with a suitable host cell.
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31. A DNA sequence which encodes the composition of matter of Claim 12.
- 30 32. A replicable expression vector capable of expressing in a suitable host cell the DNA sequence of Claim 13.
33. A DNA sequence which encodes the composition of matter of Claim 14.
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34. A replicable expression vector capable of expressing in a suitable host cell the DNA sequence of Claim 33.
- 5 35. A DNA sequence which encodes for the amino acid sequence of Claim 18 or 23.
36. A replicable expression vector capable of expressing in a suitable host cell the DNA sequence of Claim 35.
- 10 37. Recombinant host cells or host cell cultures transformed with the vector of Claim 29 or 30.
38. Recombinant host cells or host cell cultures transformed with the vector of Claim 32.
- 15 39. Recombinant host cells or host cell cultures transformed with the vector of Claim 34.
40. Recombinant host cells or host cell cultures transformed with the vector of Claim 36.
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41. The recombinant host cells of Claim 37-40 which are microbial host cells.
- 25 42. A method for preparing immunoglobulins in recombinant host cells which method comprises
- a) preparing a DNA sequence encoding heavy chain,
- 30 b) inserting the sequence of a) into a replicable expression vector operably linked to a suitable promoter,
- c) preparing a DNA sequence encoding light chain,
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- d) inserting the sequence of c) into a replicable expression vector operably linked to a suitable promoter,
- 5 e) transforming host cell culture with the vector of b) and host cell culture with the vector of d),
- f) recovering light chain and heavy chain from cell culture,
- 10 g) reconstituting light and heavy chain,
wherein steps f) and g) may be performed either sequentially in either order, or simultaneously.
- 15 43. The method of Claim 42 wherein the vector of b) and the vector of d) are transformed into the same host cell culture.
- 20 44. The method of Claim 43 wherein the sequence of a) and the sequence of c) are inserted into the same replicable expression vector.
- 25 45. The method of Claim 42 wherein the DNA sequence of a) encodes mammalian heavy chain, and the DNA sequence of c) encodes mammalian light chain; and wherein both DNA fragments encode amino acid sequences of the same mammalian antibody.
- 30 46. The method of Claim 42 wherein the DNA fragment of a) encodes a chimeric hybrid heavy chain and the DNA sequence of c) encodes a chimeric light chain.
- 35 47. A method for preparing Fab protein in recombinant host cells which method comprises
a) preparing a DNA sequence encoding the Fab region of heavy chain,

- b) inserting the sequence of a) into a replicable expression vector operably linked to a suitable promoter,
- 5 c) preparing a DNA sequence encoding light chain,
- d) inserting the sequence of c) into a replicable expression vector operably linked to a suitable promoter,
- 10 e) transforming host cell culture with the vector of b) and host cell culture with the vector of d).
- f) recovering light chain and Fab protein of heavy chain from cell culture,
- 15 g) reconstituting light and heavy Fab region chains;
wherein steps f) and g) may either be performed sequentially in either order or simultaneously.
48. The method of Claim 47 wherein the vectors of b) and d) are
20 transformed into the same host cell culture.
49. A method for preparing univalent antibody in recombinant host cells which method comprises
- 25 a) preparing a DNA sequence encoding heavy chain,
- b) inserting the sequence of a) into a replicable expression vector operably linked to a suitable promoter,
- c) preparing a DNA sequence encoding light chain,
- 30 d) inserting the sequence of c) into a replicable expression vector operably linked to a suitable promoter,

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- e) preparing a DNA sequence encoding the Fc portion of heavy chain
- 5 f) inserting the sequence of e) into a replicable expression vector operably linked to a suitable promoter,
- 10 g) transforming host cell culture with the vector of b) host cell culture with the vector of d), and host cell culture with the vector of f)
- 15 h) recovering light chain, heavy chain, and Fc portion of heavy chain from cell culture,
- i) reconstituting light chain, heavy chain, and Fc portion of heavy chain
15 wherein steps h) and i) may be performed sequentially in either order or simultaneously.
50. The method of Claim 49 wherein the vectors of b), d) and f) are transformed into the same host cell culture.
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51. A method for preparing heavy chain or light chain which method comprises
- 25 a) preparing a DNA sequence encoding heavy or light chain,
- b) inserting said sequence into a replicable expression vector operably linked to a suitable promoter,
- 30 c) transforming host cell culture with the vector of b) and
- d) recovering heavy or light chain from cell culture.

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52. A method for preparing Fab region of heavy chain as a polypeptide which method comprises

a) preparing a DNA sequence encoding Fab region of heavy chain,

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b) inserting said sequence into a replicable expression vector operably linked to a suitable promoter,

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c) transforming host cell culture with the vector of b),

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d) recovering Fab region of heavy chain from cell culture.

add B' add C' add E' add G'

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add H'

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add I'

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